Standard Operating Procedure for Extraction and Determination of Chlorinated Herbicides in Fish Tissue

1.0 Scope and Application

1.1 This is a gas chromatographic (GC) method applicable for the extraction and determination for the listed analytes. This method is for the extraction of the analytes in soil or sediment. This method should be used by, or under the supervision of experienced analysts. The analyst should be skilled in liquid-liquid extractions, derivation procedures, and the use of GC and interpretation of gas chromatograms.

1.2 Target compounds that can be measured using this method include the following:

Analyte	CAS #
Acifluorfen	50594-66-6
Bentazon	25057-89-0
Bromoxynil	1689-84-5
4-Chloro-o-tolyoxyacetic acid (MCPA)	94-74-6
2,4-D	94-75-7
2,4-DB	94-82-6
Dacthal (Chlorthal)	1861-32-1
Dicamba	1918-00-9
3,5-Dichlorobenzoic acid(3,5-DCBA)	51-36-5
Dichlorprop	120-36-5
Dinoseb	88-85-7
Hoelon (Diclofop)	40843-25-2
Pentachlorophenol (PCP)	87-86-5
Picloram (Tordon)	1918-02-01
2,4,5-T	93-76-5
2,4,5-TP (Silvex)	93-72-1

1.3 This method is applicable to the determination of the target analytes over the ranges of 0.06-37.5µg/g. The method detection limits (MDLs) are determined by the standard concentrations and the size of the sample used.

1.4 The following compounds may require some special attention when being determined by this method:

1.4.1 2,4-DB co-eludes with Dinoseb on the RTX-5ms column. Since the RTX-5 column is the confirmation column this problem can be corrected by

analyzing the sample on GC/MS system.

1.4.2 The analytes from this method are extracted as carboxyl acids, which breakdown in the injection port of the GC. To correct this problem the analytes are derivatized first with diazomethane.

2.0 Summary of Method

The analytes are extracted from the solid material first by sonication, were they under go hydrolysis. After hydrolysis, the analytes are acidified and extracted from the water and lipids with methylene chloride. After extraction the analytes are derivatized into the methyl esters and analyzed by gas chromatography.

3.0 Definitions

The definitions and purposes below are specific to this method, but have been conformed to common usage as much as possible.

3.1 GC/ECD - gas chromatograph/electron capture detector

3.2 Reagent water - Reagent water is defined as a water in which an interference is not observed > the EDL of each analyte of interest. A Millipore or Barnstead water system or its equivalent may be used to generate deionized reagent water. Distilled water that has been passed through granular charcoal may also be suitable. Reagent water is monitored through analysis of the laboratory reagent blank.

3.3 Quality control sample (QCS) - A solution of method analytes of known concentration which is used to fortify an aliquot of reagent water or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.4 Laboratory fortified blank (LFB) - A blank fish to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.5 Laboratory reagent blank (LRB) - A blank solid sample that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is

used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.6 Laboratory fortified matrix (LFM) - Spiked sample - An environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.7 Laboratory duplicate - Two aliquots LD1 and LD2 of the same designated as such in the laboratory. Each aliquot is extracted, derivatized and analyzed separately with identical procedures. Analysis of LD1 and LD2 indicate the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.8 Target compound - An analyte of compound listed in section 1.2

3.9 Surrogate analyte - A pure analyte that is chemically similar to the target compounds but is not expected to occur in an environmental sample. It is added to a sample aliquot in a known amount before extraction and is measured with the sample procedures used to measure other sample components. The purpose of the surrogate is to monitor method performance with each sample.

3.10 Spiking solution - A mixture of analytes listed in section 1.2. These compounds are added to the LFB and LFM in known amounts. The spiking solution is added before extraction to measure the response of the method analytes and surrogates.

3.11 Stock Standard solution (SSS) - A concentrated solution one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.12 Primary dilution standard solution - A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.13 Calibration standards (CAL) - A solution prepared from the primary dilution standard solution and stock standards solutions of the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument

response with respect to analyte concentration.

3.14 Continuing calibration check (CCC) - A calibration standard containing one or more method analytes which is analyzed periodically to verify the accuracy of the existing calibration curves or response factors for those analytes.

3.15 Tube spike and surrogate - Laboratory performance check sample - A solution of method analytes and surrogate in solvent that is used to evaluate the performance of the instrumental system with respect to a defined set of method criteria.

3.16 Method detection limit (MDL) - the MDL is defined as the minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence.

3.17 Material safety data sheet (MSDS) - Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire and reactivity data including storage, spill, and handling precautions.

3.18 MTBE - Methyl-tert-butyl-ether- The final solvent the extracts are in.

3.19 Micrograms per gram (ug/g) - The final units for the concentrations of the extracted analytes.

3.20 Definitions of a few terms in the method:

3.20.1 May: This action, activity, or procedural step is neither required nor prohibited

3.20.2 May not: This action, activity, or procedural step is prohibited.

3.20.3 Must: this action, activity, or procedural step is required.

3.20.4 Shall: This action, activity, or procedural step is required.

3.20.5 Should: This action, activity, or procedural step is suggested, but not required.

3.21 Estimated Detection Limit (EDL) - Defined as either the MDL or a level of a compound in a sample yielding a peak in the final extract that can be identified, measured, and reported with 99% confidence that the concentration is greater than zero.

4.0 Interferences

4.1 Method interferences may be caused by contaminations in solvents, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baselines in chromatograms. All reagents as apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by analyzing laboratory reagent blanks as described in section 9.0. Subtracting blank values from sample results is not permitted.

4.1.1 Glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with dilute acid, tap, and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 1 hour. Do not heat volumetric ware. Thermally stable materials such as PCBs might not be eliminated by this treatment. Thorough rinsing with acetone and hexane may be substituted for the heating. After drying and cooling, store glassware in a clean environment free of all potential contamination. To prevent any accumulation of dust or other contaminants, store glassware inverted or capped with aluminum foil.

4.2 The use of high purity reagents and solvents helps to minimize interference problems. Each new bottle of solvent should be analyzed before use. An interference free solvent is a solvent containing no peaks yielding data at $\geq$ MDL and at the retention times of the analytes of interest. Purification of solvents by distillation in an all glass system may be required.

4.3 Matrix inferences may be caused by contaminations and lipids that are co-extracted from the sample. This is a major problem when analyzing samples of this method. This method tries to eliminate as much interferences as possible with the extraction process and GC oven programing. However, analyte identification should be confirmed using the confirmation column.

4.4 Analytical bias may result from discrimination at the GC inlet. This can be minimized by optimizing the inlet configuration and injection technique.

4.5 The acidic form of the analytes are strong organic acids which react readily with alkaline substances and can be lost during sample preparation. Glassware and glass wool must be acid-rinsed with 1N hydrochloric acid and the sodium sulfate may be acidified with sulfuric acid prior to use to avoid analyte losses due to adsorption.

4.6 Organic acids and phenols, especially chlorinated compounds, cause the most direct interference with the determination. Alkaline hydrolysis and subsequent extraction of the basic sample removes many chlorinated hydrocarbons and phthalate esters that might otherwise interfere with electron capture analysis.

4.7 Interferences by phthalate esters can pose a major problem in pesticide analysis when using the ECD. These compounds generally appear in the chromatogram as large peaks. Common flexible plastics contain varying amounts of phthalate, that are easily extracted or leached during laboratory operations. Cross contamination of clean glassware routinely occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled. Interferences from phthalate can best be minimized by avoiding the use of plastic in the laboratory. Exhaustive purification of reagents and glassware may be required to eliminate background phthalate contamination.

4.8 It is important that samples and working standards be contained in the same solvent. The solvent for working standards must be the same as the final solvent used in sample preparation. If this is not the case, chromatographic comparability of standards to sample may be affected.

5.0 Safety

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. Accordingly, exposure to these chemicals must be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of MSDSs should also be made available to all personnel involved in the chemical analysis.

5.2 DIAZOMETHANE - A toxic carcinogen which can explode under certain conditions. The following precautions must be followed:

5.2.1 Use only a well ventilated hood - do not breath vapors.

5.2.2 Use a safety screen

5.2.3 Use mechanical pipetting aides

5.2.4 Do not heat above 90C - **EXPLOSION** may result.

5.2.5 Avoid grinding surfaces, ground glass joints, sleeve bearings, glass stirrers - **EXPLOSION** may result.

5.2.6 Store away from alkali metals - **EXPLOSION** may result.

5.2.7 Solutions of diazomethane decomposes rapidly in the presence of solid materials such as copper powder, calcium chloride, and boiling chips.

5.2.8 The diazomethane generation apparatus used in the esterification procedures (Sect. 11.4) produces micro molar amounts of diazomethane to minimize safety hazards.

5.3 WARNING - When a solvent is purified, stabilizers added by the manufacturer are removed, thus potentially making the solvent hazardous.

6.0 Equipment and Supplies

Note: Brand names, suppliers, and part numbers are cited for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

6.1 Glassware

6.1.1 Cleaned glass container with Teflon lined screw top (sample collection and storage of dried samples)

6.1.2 200mL glass centrifuge bottles with Teflon lined screw top - 1 per sample

6.1.3 150mL (or larger) beaker - one per sample

6.1.4 200mL graduated cylinder or other device for measuring 50mL and 150mL aliquots. May use one and rinse with reagent water between samples or 1 per sample.

6.1.5 Separatory funnel - 1000mL or 2000mL, with TFE-fluorocarbon stopcocks, ground glass or TFE-fluorocarbon stoppers.

6.1.6 Turbo vap collection tubes, 200mL maximum capacity - 1 per sample

6.1.7 15 ml graduated centrifuge tubes - 1 per sample

6.1.8 Hewlett Packard autosampler vials with Teflon lined tops.

6.2 Grinder/blender - Capable of grinding the dried sample to a fine powder for homogenous sample for extraction.

6.3 Sonicator - Capable of holding all the samples while in 200mL centrifuge tubes.

6.4 Lab shaker - Capable of variable speeds and capacity of holding all the samples while in 200mL centrifuge tubes.

6.5 40-50mL centrifuge tubes with covers, plastic may be used - 2 per sample

6.6 Centrifuge - Capable of holding the 50mL centrifuge tubes and reach a speed of 700rpm.

6.7 Balance - Analytical, capable of accurately weighing to the nearest 0.01g

6.8 Diazomethane generator - Assemble from two 20x150 mm test tubes, two Neoprene rubber stoppers, and a source of nitrogen as shown in Figure 1. When esterification is performed using diazomethane solution, the diazomethane collector is cooled in an approximately 2-L thermos for ice bath or a cryogenically cooled vessel.

6.9 Zymarck Turbo Vap II or equivalent evaporation device.

6.10 Hewlett Packard 5890 Gas Chromatograph with data system

6.10.1 The GC must be capable of temperature programming and be equipped with a split less injector.

6.10.2 GC supplies including injection port liners, ferrules, syringes etc...

6.10.3 The gas chromatograph must be equipped with an electron capture detector

6.10.4 An auto-injector is recommended for improved precision of analysis.

6.10.5 The interfaced data system is the LabSystems Xchrom or an equivalent system which allows for data acquisition, storage, retrieval, and

calculations of analytes. (See the manual for details)

6.10.6 Capillary column 30 meters long, 0.25mm ID, 0.25 micron film thickness or equivalent.

6.11 Freeze dry apparatus - This is used to freeze dry the samples, thus preserving the fish tissue longer.

7.0 Reagents and Standards

7.1 Acetone, Hexane, Methanol, Methylene Chloride, MTBE, and Ethyl Ether - Optima grade or nanograde or distilled in glass or in other words the highest purity to reduce any interference problems. The residue grade solvents are flammable and stored in appropriate flammable storage in the solvent vault located off the chemical storage room. Reagent grade inorganic chemicals shall be used in all tests.

7.2 Sodium sulfate, granular, anhydrous, ACS grade - May be heat treated in a shallow tray at 450C for a minimum of 4 hours to remove interfering organic substances.

7.3 Sodium Hydroxide (NaOH), pellets - ACS grade

7.3.1 Dissolve 13.3g NaOH in 1L reagent water

7.4 Phosphoric Acid, concentrated - ACS grade

7.5 Saturated sodium chloride solution

7.5.1 Sodium chloride (NaCl), crystal, ACS grade - Heat treat in a shallow tray at 450C for a minimum of 4 hours to remove interfering organic substances.

7.5.2 Dissolve heat treated NaCl in reagent water. Add NaCl to solution until no more salt can be dissolved into solution.

7.6 Carbitol (Diethylene Glycol Mono Ethyl ether) - ACS grade
Available from Aldrich Chemical Co.

7.7 Diazald, ACS grade - Available from Aldrich Chemical Co.

7.8 Diazald solution - Prepare a solution containing 5 g Diazald in 50mL of a 50:50 by volume mixture of ethyl ether and Carbitol. This solution is stable for 3 months when stored at 4C in an amber bottle with a Teflon-lined screw cap.

7.9 2,4-Dichlorophenylacetic acid (2,4-DCAA) - 99% purity, for use as surrogate standard. Available from Chemservice and Aldrich Chemical Co. 2,4-DCAA must be in the acidic form.

7.9.1 125ug/ml solution is used as surrogate: prepare by pipetting 625uL of stock solution (2000ug/ml) into 10mL volumetric flask. Fill the flask to mark with MTBE.

7.10 Silicic Acid, ACS grade

7.11 Reference standards - Supplied by Restek or equivalent. The calibration standards must be from a different source than the spiking solution. Stock standard solutions are stored in the vials in the organic lab refrigerator until ready to be diluted to the calibration standards. Calibration standards should be checked frequently for signs of evaporation or degradation. Table 1 shows the concentrations of method analytes in the stock and calibration solutions. All analytes in calibrations standards must in the methyl ester form.

7.12 Spiking solution - Supplied by Chemserve or equivalent. The spiking solution must be from a different source than the reference standards. Table 2 shows the concentration of the method analytes in the spiking solution. All analytes in the spiking solution must be in the acid form.

7.13 Potassium Hydroxide (KOH) pellets - ACS grade

7.13.1 37% KOH (w/v) - Dissolved 37g KOH pellets in reagent water and dilute to 100mL.

7.14 Hydrochloric acid (HCl) for rinsing glassware:

7.14.1 400mL concentrated HCl in 4L reagent water.

8.0 Sample collection, preservation, and storage

8.1 Fish samples should be chopped or ground up for easier handling. After chopping place the samples in 0C or colder to freeze the fish.

8.2 After freezing, the fish samples should be freeze dried to increase the shelf life of the sample and for easier extractions.

8.3 Grind the freeze dried samples for a more homogeneous extraction.

8.4 Dried and ground samples should be stored in clean glass containers with Teflon lined caps.

9.0 Quality Control

9.1 Minimum quality control (GC) requirements are initial demonstration of laboratory capability, determination of surrogate compound recoveries in each sample and blank, analysis of laboratory reagent blanks, laboratory fortified samples, laboratory fortified blanks, and QC samples.

9.2 The analyst is permitted to modify the GC columns, GC conditions, detectors, continuous extraction techniques, concentration techniques, internal standards or surrogate compounds.

9.3 Laboratory reagent blanks (LRB) - Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of samples is extracted or reagents are changed, a LRB must be analyzed. If within the retention time window of any analyte the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples.

9.4 Assessing laboratory performance - Laboratory fortified blank (LFB)

9.4.1 The laboratory must analyze at least one LFB sample with every sample set. The concentration of each analyte should be 10 times the EDL. Calculate accuracy as percent recovery.

9.4.2 The control limits are developed from the mean percent recovery ($\bar{x}$) and standard deviation (S) of the percent recovery. This data is used to establish upper and lower control limits as follows:

Upper control limit = $\bar{x}$ + 3S

Lower control limit = $\bar{x}$ - 3S

After each five to ten new recovery measurements, new control limits should be calculated using only the most recent 20-30 data points. See table 3 for control limits.

9.4.3 It is recommended that the laboratory periodically determine and document its detection limit capabilities for the analytes of interest.

9.5 Laboratory Fortified sample matrix - Spiked Sample

9.5.1 The laboratory must add the spiking solution to a minimum of one sample per sample set.

9.5.2 Calculate the percent recovery, P, of the concentration for each analyte, after correcting the analytical result, X, from the fortified sample for the background concentration, b, measured in the unfortified sample.

$$P = 100(X-b)/\text{fortifying concentration}$$

9.5.3 If the recovery of any such analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control(Sect. 9.6), the recovery problem encountered with the fortified sample is judged to be matrix related, not system related. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effect.

9.6 Assessing instrument system - Laboratory performance check sample- Instrument performance should be monitored on a daily basis by analysis of the LPC sample. The LPC sample contains compounds designed to indicate appropriate instrument sensitivity, column performance (primary column) and chromatographic performance. Inability to demonstrate acceptable instrument performance indicated the need for reevaluation of the instrument system.

10.0 Calibration and Standardization

10.1 Establish GC operating parameters equivalent to those indicated in Sect. 6.10. The system should be calibrated using the following techniques. **Note:** Calibration standard solutions must be prepared such that no unresolved analytes are mixed together.

10.2 External calibration-

10.2.1 Prepare calibration standards at a minimum of three concentration levels for each analyte and surrogate compound. Table 1 shows how to prepare the standards. All standard must be in MTBE and the methyl ester of the analytes. The lowest standard should represent analyte concentrations at the EDL. The remaining standards should bracket the analyte

concentrations expected in the samples and should define the working range of the detector.

10.2.2 Starting with the standard of lowest concentration, analyze each calibration standard according to sect. 11.5 and tabulate response area versus the concentration in the standard. The results can be used to prepare a calibration curve for each compound. The instrument's data processing software may be used to help calculate the calibration curve.

10.2.3 The working calibration curve must be verified on each working day by the measurement of a minimum of two calibration check standards, one at the beginning and one at the end of the analysis day.

10.2.4 Single point calibration is a viable alternative to a calibration curve. Prepare a single point standard at a concentration that produces a response that deviates from the sample extract response by no more than 20%.

10.2.5 Verify calibration standards periodically by analyzing a standard prepared from reference material obtained from an independent source.

11.0 Procedure

11.1 Hydrolysis, Preparation

11.1.1 Weigh 2 grams ($\pm$0.1g) of the dried and ground sample in to a 200ml centrifuge bottle. Include an LRB, LFB, matrix sample spike, and sample duplicate.

11.1.2 Fortify each sample with 100uL of 125ug/ml 2,4-DCAA surrogate.

11.1.3 Spike the LFB and sample spike with 150uL of spiking solution, see table 2 for concentration of analytes.

11.1.4 Add 150mL of 13.3g/L NaOH solution to each sample. Seal and shake to mix. Check the pH of sample with pH paper; if the sample does not have a pH greater than or equal to 12, adjust the pH by adding more NaOH solution. **Note:** Be sure to make note of the extra volume of NaOH added, it will affect the calculations during analysis.

11.1.5 Sonicate the sealed samples in lab sonicator for 15 minutes. **Note:** Shake the samples every 5 minutes to break the bead of fish in the container to

aid in the sonication.

11.1.6 Shake the samples for 1 hour in automated lab shaker. The bottles should be secured so that they do not break while in the shaker.

11.2 Acidification and extraction

11.2.1 Decant 60-100mL of sample in to a small beaker.

11.2.2 Add 5mL concentrated phosphoric acid and stir to mix.

11.2.3 Pour the acidified sample in to two clean centrifuge tubes. Plastic tubes may be used for the centrifuge. The time the sample is in the tubes will not effect the sample or create unmanageable interferences.

11.2.4 Centrifuge the samples at 700-800 rpms for 10 minutes or until all the precipitate has been separated from the aqueous phase.

11.2.5 To separatory funnel add: 100mL saturated NaCl water and 50mL aliquot of acidified sample.

11.2.6 Add 60mL methylene chloride to the separatory funnel and extract the sample by vigorously shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase. Emulsions will form due to the lipids that are present from the fish samples. The analyst should use the following to break the emulsion to the best of their ability. If the emulsion interference between layers is more that 1/3 of the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends on the sample, but may include centrifugation, filtration or other physical methods. Collect the methylene chloride (bottom) phase in the turbo vap evaporation vessels. **Note:** Care should be taken to ensure that there is no water in the collected methylene chloride. Water will interfere with the esterification process, thus affecting the recovery of the analytes.

11.2.7 Add a second 60mL volume of methylene chloride to the separatory funnel and repeat extraction procedure a second time, collecting the methylene chloride layer.

11.2.8 Perform the third extraction in the same manner.

11.3 Extract concentration

11.3.1 Exchange solvent of extracts to MTBE.

11.3.1.1 Evaporate samples to about 1 ml and rinse the sides of container with MTBE.

11.3.1.2 Repeat evaporation and rinsing with MTBE for a total of 3 times.

11.3.2 Analytically transfer exchanged samples to 15mL graduated centrifuge tubes. Evaporate samples under a flow of nitrogen to 1mL.

11.4 Esterification of acids - See Sect. 5.2 for safety precautions

11.4.1 Assemble the diazomethane generator (figure 1) in a hood. The collection vessel is a 10 or 15mL vial, equipped with a Teflon-lined screw cap and maintained at 0-5°C.

11.4.2 Add a sufficient amount of ethyl ether to tube 1 to cover the first impinger. Add 10mL of MTBE to the collection vial. Add 4mL Diazald solution and 4mL of 37% KOH solution (Sect7.8) to the second impinger. Connect tubing as shown and allow the nitrogen flow (5-10mL/min) to purge the diazomethane from the reaction vessel into the collection vial for 30 min or until the color is gone from tube 2. Cap the vial when collection is complete and maintain at 0-5C. When stored at 0-5C this diazomethane solution may be used over a period of 48 hr.

11.4.3 Make up the LRC samples.

11.4.3.1 Add 1mL MTBE to two clean graduated centrifuge tubes

11.4.3.2 To tube 1, tube spike, add the spiking solution at the same concentration as the samples. See Sect.11.1.3

11.4.3.3 To tube 2, tube surr, add the surrogate solution at the same concentration as the samples. See Sect 11.1.2

11.4.4 To each sample in graduated centrifuge tubes add 250µL methanol and 500µL diazomethane solution (Sect. 11.4.2). Samples should turn yellow after addition of the diazomethane solution and remain yellow for at least

2 min. Repeat methylation procedure if necessary.

11.4.5 Seal tubes with Teflon lined caps. Mix well and store at room temperature in a hood for at least 30 min.

11.4.6 Destroy any unreacted diazomethane by adding 0.1 to 0.2 grams silicic acid to the centrifuge tubes. Mix well and adjust the sample volume to 5.0mL with MTBE.. Allow to stand until the evolution of nitrogen gas has stopped (approximately 20 min).

11.4.7 Transfer about 1mL of sample to autosampler vials, seal, and analyze by GC/ECD.

11.5 Gas Chromatograph

11.5.1 Sect. 6.10 summarizes the recommended operating conditions for the GC. Included in table 4 are retention times observed using this method. Other GC columns, chromatographic conditions, or detectors may be used.

11.5.2 Calibrate the system daily as described in section 10.0. The standards and extracts must be in MTBE.

11.5.3 Inject 2µL of the sample extract. Record the resulting peak size in area units and the retention times of the peaks. The instrument's data processing system may be used to record the data.

11.5.4 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

11.6 Identification of analytes

11.6.1 Identify a sample component by comparison of its retention time to the retention time of a the standard chromatogram. If the retention time of an unknown compound corresponds, within limits, to the retention time of a standard compound, then the identification is considered positive.

11.6.2 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily

in the interpretation of chromatograms.

11.6.3 True identification of the analytes requires confirmation on a dissimilar column (see section 6.10). When analytes appear to not be resolved chromatographically on 2 columns the GC/MS may be used to positively identify the components of the sample.

12.0 Calculations and reporting results

The instrument's data collection software may be used to calculate the results instead of the manual calculations shown below.

12.1 Calculate analyte concentrations in the sample from the response for the analyte using the calibration procedure described in Sect 10.

12.2 Calculate the amount of material injected from the peak response using the calibration curve determined sect 10.2. The concentration (C) in the sample can be calculated from following equation.

$$C(ug/L) = \frac{(A)(Vt)}{(Vi)(Vs)}$$

A = Amount of material injected (ng) (found from the calibration curve)
Vi = Volume of extract injected (uL) (2uL)
Vt = Volume of total extract (uL) (5mL = 5000)
Vs = Volume of water extracted (mL)(1000mL)

12.3 Values for each analyte should be reported in ug/g. To calculate this use the following equation:

$$C(ug/L) \times 3)/Wi = R(ug/g)$$

R = concentration of analyte in ug/g
C = concentration calculated in 12.2 (ug/L)
Wi = weight of solid sample used in 11.1.1 (g)
The 3 is present because originally there was 150mL of water (Sect 11.1.4) to be extracted but there was only 50mL extracted in 11.2.5.

12.4 The results for each analyte should be reported in ug/g as per a dry weight basis.

13.0 Method Performance

There is data to show that this method is accurate and precise. Table 5 shows some

results for this method.

14.0 Pollution Prevention

14.1 The large volume of organic solvent is a potential for pollution. The analyst should take care to properly use the solvent in vented hoods. The excess solvent can be placed in hoods to evaporate or recycled.

15.0 Waste Management

15.1 The volume of organic solvent should have a plan of action for waste management. The soil sample can be disposed in the garbage, water can be diluted and down the drain, and the organic solvent can be recycled or evaporated in to a vented hood.

15.2 For further information on waste management consult The Waste Management Manual for Laboratory Personnel and Less is Better: Laboratory Chemical Management for Waste Reduction, both available from the American Chemical Society's Dept of Government Relations and Science Policy, 1155 16th Street NW, Washington, DC 20036.

16.0 References

16.1 EPA Method 515.1, Revision 4.0, R.C. Dressman, J.J. Lichtenberg, J.W. Hodgeson, T. Engels, and R.L. Graves

17.0 Tables and graphs

Table 1: Calibration Standard Concentrations

Concentration for each analyte in Calibration Standards
All analytes are in the methyl ester form
The amount of stock solution diluted into 5mL of MTBE is listed for each standard

Analyte	Stock Solution	Standard 1 (MDL) 20µL	Standard 2 40µL	Standard 3 200µL	Standard 4 400µL	Standard 5 600µL
3,5 DCBA	12.5µg/mL	0.05µg/mL	0.1µg/mL	0.5 µg/ml	0.75µg/mL	1.5µg/mL
2,4-DCAA	25.0	0.1	0.2	1.0	1.5	3.0
Dicamba	5.0	0.02	0.04	0.2	0.3	0.6
MCPA	1250.0	5.0	10.0	50.0	75.0	150.0
Dichlorprop	15.0	0.06	0.12	0.6	0.9	1.8
2,4-D	10.0	0.04	0.08	0.4	0.6	1.2
Bromoxynil	2.5	0.01	0.02	0.1	0.15	0.3

PCP	2.0	0.008	0.016	0.08	0.12	0.24
2,4,5-TP	5.0	0.02	0.04	0.2	0.3	0.6
2,4,5-T	5.0	0.02	0.04	0.2	0.3	0.6
Dinoseb	10.0	0.04	0.08	0.4	0.6	1.2
2,4-DB	25.0	0.05	0.1	0.2	0.5	1.0
Bentazon	25.0	0.1	0.2	1.0	1.5	3.0
Dacthal	2.5	0.01	0.02	0.1	0.15	0.3
Tordon	5.0	0.02	0.04	0.2	0.3	0.6
Acifluorfen	10.0	0.04	0.08	0.4	0.6	1.2
Hoelon	25.0	0.1	0.2	1.0	1.5	3.0

Table 2: Spiking solution concentration

Concentration of analytes in Spiking solution
All analytes must be in the acid form
Solvent: MTBE

Analyte
3,5-DCBA
Dicamba
MCPA
Dichlorprop
2,4-D
Bromoxynil
PCP
2,4,5-TP
2,4,5-T
Dinoseb
2,4-DB
Bentazon
Dacthal
Tordon
Acifluorfen
Hoelon

Concentration
125µg/mL
50
12500
150
100
25
20
50
50
100
250
250
25
50
100
250

Table 3: Upper and Lower Control Limits

The Percent Recovery the analytes in the LFB, LRB, and spike should be between in order to pass the run.

Analyte	Lower Limit	Upper Limit
3,5-DCBA	53.1%	150.9%
2,4-DCAA	70.0%	130.0%
Dicamba	37.8%	232.4%
MCPA	NA	NA
Dichlorprop	46.1%	167.9%
2,4-D	48.5%	213.5%
Bromoxynil	NA	NA
PCP	36.4%	223.6%
2,4,5-TP	41.6%	226.4%
2,4,5-T	67.8%	166.2%
Dinoseb	-0.9%	84.9%
2,4-DB	NA	NA
Bentazon	69.6%	170.4%
Dacthal	NA	NA
Tordon	44.5%	137.5%
Acifluorfen	73.9%	168.1%
Hoelon	NA	NA

Table 4: Guide to retention times

Retention times and order found on the columns
NOTE: These times are meant to be a guide only

Analyte	Primary Column RTX-50ms	Confirmation Column RTX-5ms
3,5-DCBA	11.14	7.88
2,4-DCAA	16.90	11.40
Dicamba	17.90	11.71
MCPA	19.81	13.55
Dichlorprop	20.87	15.03
2,4-D	22.99	15.91
Bromoxynil	23.24	15.42
PCP	23.97	18.14
2,4,5-TP	25.78	19.86
2,4,5-T	28.18	20.98
Dinoseb	29.31	23.39
2,4-DB	29.87	23.48
Bentazon	33.72	23.96
Dacthal	34.57	27.41
Tordon	37.37	25.64
Acifluorfen	42.37	35.63
Hoelon	48.92	39.87

Injector @ 235ºC, Detectors @ 300C
Oven Program: Hold at 100ºC for 1 minute
Ramp to 226ºC at 3ºC/min, hold 12 minutes
Ramp to 286ºC at 30ºC/min, hold 10 minutes

Table 5:

Analyte Accuracy and Precision Data[1]

Analyte	Fortified Conc µg/L	mean meas conc µg/L	std dev µg/L %	EDL[2] µg/L	EDL[3] µg/g
3,5-DCBA	6.25	4.22	1.26	0.25	0.038
2,4-DCAA	4.16	2.44	0.76	0.5	0.075
Dicamba	2.50	2.08	0.36	0.1	0.015
MCPA	625.00	452.60	109	25.0	3.75
Dichlorprop	7.50	5.69	1.54	0.3	0.045
2,4-D	5.00	3.95	1.03	0.2	0.03
Bromoxynil	1.25	1.04	0.36	0.05	0.008
PCP	1.00	0.49	0.21	0.04	0.006
2,4,5-TP	2.50	1.42	0.41	0.1	0.015
2,5,6,-T	2.50	5.87	8.08	0.1	0.015
Dinoseb	5.00	2.29	1.07	0.2	0.03
Bentazon	12.50	7.15	2.61	0.5	0.075
Dacthal	1.25	1.01	0.52	0.05	0.008
Tordon	2.50	2.19	0.4	0.1	0.015
Acifluorfen	5.00	7.85	4.16	0.2	0.03
Hoelon	12.50	10.05	2.18	0.5	0.075

[1]Produced by analysis of five fortified fish samples

[2]Estimated detection limit

[3]Estimated detection limit EDL(µg/L)*3/20 = EDL(µg/g)

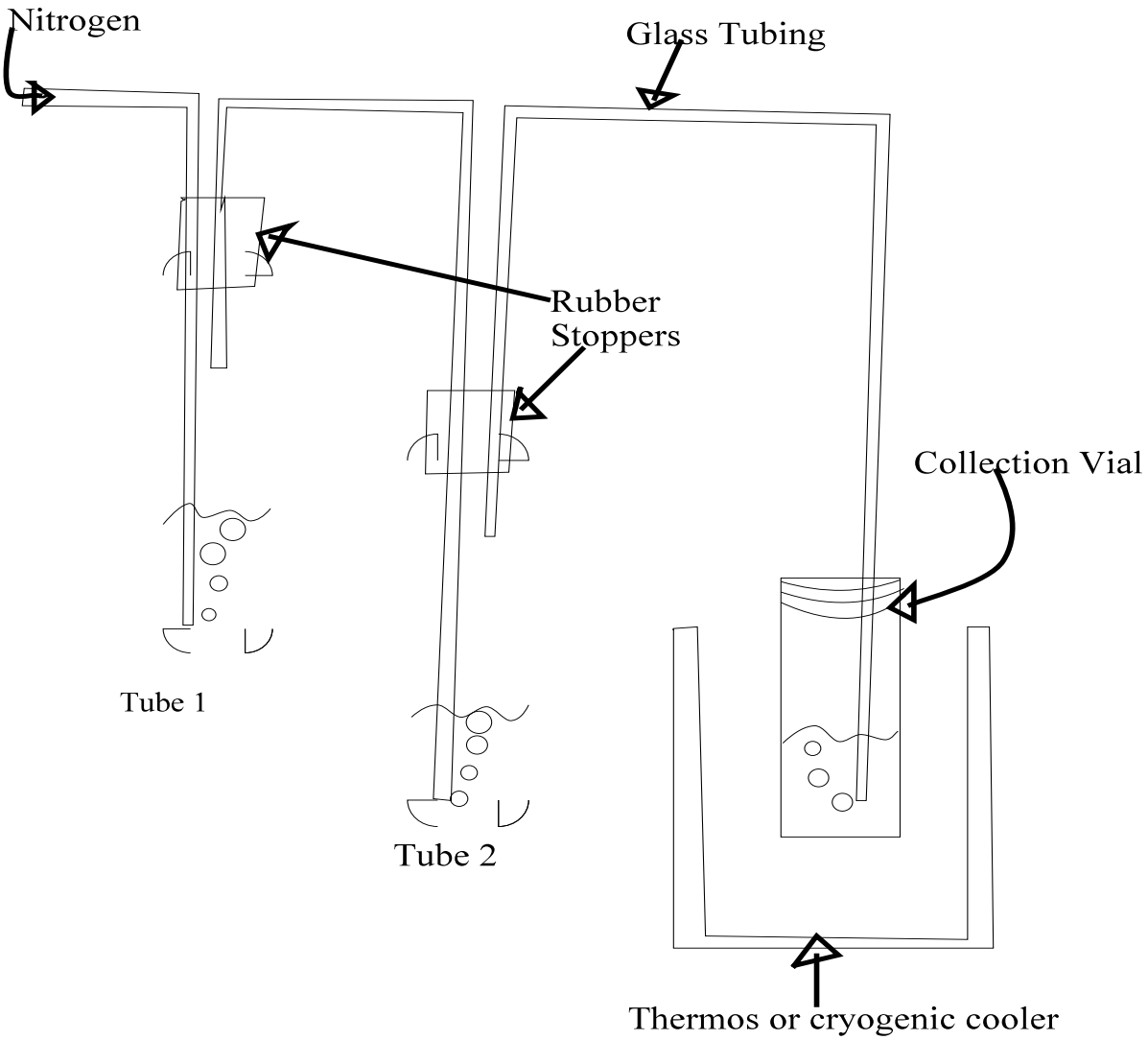

Figure 1: Diazomethane Solution Generator